Amendments to the Specification

In the Specification, please replace the paragraph beginning on page 5, line 25 with the following amended paragraph:

To assess the efficacy of drugs in the treatment of patients *in vivo*, clinical markers of virus replication needed to be defined. In the past, some surrogate markers, like CD-cell count, have been used. More recently, some commercial assays like Quantiplex (Chiron), NucliSense (Organon-Teknika) and ~~Amplicor HIV-1 Monitor~~ AMPLICOR HIV-1 MONITOR™ (Roche) were developed to directly measure viral load. These viral load determinations proved to be an excellent tool in monitoring therapeutic efficiency for HAART and for clinical trials with new experimental drugs.

Please replace the paragraph beginning on page 19, line 7 with the following amended paragraph:

Figure 1 is an illustration of a calibration of standard curve for HIV-1 (**1a**), HCV (**1b**), BVDV (**1c**), mitochondrial DNA (**1d**) and molecular toxicology (**1e**) RT-PCR. The attenuated clinical samples were diluted in DMEM-F12/10% FBS. The Ct value indicates the threshold cycle where the one-step RT-PCR detection of the target becomes positive. The Log cp/mL value is the logarithm of the amount of target copies per mL sample. The ◆ line indicates the Roche ~~Amplicor HIV-1 Monitor~~ AMPLICOR HIV-1 MONITOR™, while the ■ line indicates real-time HIV-1 RT-PCR.

Please replace the paragraph beginning on page 33, line 2 with the following amended paragraph:

For the detection of host nucleic acids, any suitable primer and/or probe known in the art may be used. These primers and/or probes may be purchase or made by any means known in the art. There are several primers and/or probe combinations commercially available, for example the primer probe set for rRNA gene (Perkin Elmer/Applied Biosystems). The latter set is often used as calibrator PCR in this invention. Alternatively, suitable probes and primers can be designed by using the ~~Primer Express~~ PRIMER EXPRESS® software (Applied Biosystems,

CA), and in particular new primers and probes for the β-actin gene, and for the mitochondrial cytochrome oxidase subunit II (COXII) gene.

Please replace the paragraph beginning on page 33, line 11 with the following amended paragraph:

In one embodiment, the nuclear DNA or RNA used to derive a set of oligonucleotides for the endogenous control is the DNA for β-actin. Any suitable primers and/or probes can be used. In a specific embodiment of the present invention, the primers and/or probes are complementary to sequences from the third exon of the human β-actin gene (GenBandk accession number E01094). The probe comprises a reporter and quencher that provides a detectable signal upon amplification. Any reporter/quencher probe set can be used, including, but not limited to ~~TaqMan~~TAQMAN®, molecular beacons, single dye probe, SYBR® green, ~~Amplifluor~~AMPLIFLUOR™ probes and dual labeled probe sets.

Please replace the paragraph beginning on page 34, line 2 with the following amended paragraph:

In one embodiment, the mitochondrial nucleic acids can be specifically derived from mitochondrial DNA. In an alternate embodiment, the mitochondiral nucleic acids can be specifically derived from mitochondrial RNA. In an alternate embodiment, the mitochondiral nucleic acids are complementary to sequences from the mitochondrial COXII gene. Any suitable primers and/or probes can be used. The probe comprises a reporter and quencher that provides a detectable signal upon amplification. Any reporter/quencher probe set can be used, including, but not limited to ~~TaqMan~~TAQMAN®, molecular beacons, single dye probe, SYBR® green, ~~Amplifluor~~AMPLIFLUOR™ probes and dual labeled probe sets.

Please replace the paragraph beginning on page 34, line 18 with the following amended paragraph:

For viral targets, any suitable primer and/or probe known in the art may be used. These primers and/or probes may be purchase or made by any means known in the art. Alternatively, suitable probes and primers can be designed by using the ~~Primer Express~~PRIMER EXPRESS® software (Applied Biosystems, CA), and in particular, primers and probes designed to be

complementary to highly conserved areas. This is particularly important for viruses with a high genetic variability, like for example HCV, HBV, and HIV, BVDV and RSV.

Please replace the paragraph beginning on page 35, line 9 with the following amended paragraph:

In one embodiment of the invention, the target viral nucleic acid is from HIV, and in particular, HIV-1. Any suitable primers and/or probes can be used. In a specific embodiment of the present invention, the primers and/or probes are complementary to the reverse transcriptase domain between codons 200 and 280. The probe comprises a reporter and quencher that provides a detectable signal upon amplification. Any reporter/quencher probe set can be used, including, but not limited to ~~TaqMan~~TAQMAN®, molecular beacons, single dye probe, SYBR® green, ~~Amplifluor~~AMPLIFLUOR™ probes and dual labeled probe sets.

Please replace the paragraph beginning on page 35, line 25 with the following amended paragraph:

In another embodiment of the invention, the target viral nucleic acid is from HCV. Any suitable primers and/or probes can be used. In a specific embodiment of the present invention, the primers and/or probes are derived from highly conserved sequences complementary to the RNA sequences present in HCV, such as the HCV 5' non-coding region. The probe comprises a reporter and quencher that provides a detectable signal upon amplification. Any reporter/quencher probe set can be used, including, but not limited to ~~TaqMan~~TAQMAN®, molecular beacons, single dye probe, SYBR® green, ~~Amplifluor~~AMPLIFLUOR™ probes and dual labeled probe sets.

Please replace the paragraph beginning on page 36, line 14 with the following amended paragraph:

In another embodiment of the invention, the target viral nucleic acid is from BVDV. Any suitable primers and/or probes can be used. In a specific embodiment of the present invention, the primers and/or probes are derived from highly conserved sequences complementary, such as sequences complementary to nucleotides 1611 to 1751 of the NS5B gene. The probe comprises a reporter and quencher that provides a detectable signal upon amplification. Any

reporter/quencher probe set can be used, including, but not limited to ~~TaqMan~~TAQMAN®, molecular beacons, single dye probe, SYBR® green, ~~Amplifluor~~AMPLIFLUOR™ probes and dual labeled probe sets.

Please replace the paragraph beginning on page 37, line 2 with the following amended paragraph:

In another embodiment of the invention, the target viral nucleic acid is from HBV. Any suitable primers and/or probes can be used. In a specific embodiment of the present invention, the primers and/or probes are derived from highly conserved sequences complementary to the DNA sequences present in HBV, such as the amino-terminal region of the HBV surface antigen gene. The probe comprises a reporter and quencher that provides a detectable signal upon amplification. Any reporter/quencher probe set can be used, including, but not limited to ~~TaqMan~~TAQMAN®, molecular beacons, single dye probe, SYBR® green, ~~Amplifluor~~AMPLIFLUOR™ probes and dual labeled probe sets.

Please replace the paragraph beginning on page 37, line 19 with the following amended paragraph:

In another embodiment of the invention, the target viral nucleic acid is from RSV. Any suitable primers and/or probes can be used. In a specific embodiment of the present invention, the primers and/or probes are derived from highly conserved sequences complementary, such as sequences complementary to nucleotides that encode for the RNA polymerase large subunit (L). The probe comprises a reporter and quencher that provides a detectable signal upon amplification. Any reporter/quencher probe set can be used, including, but not limited to ~~TaqMan~~TAQMAN®, molecular beacons, single dye probe, SYBR® green, ~~Amplifluor~~AMPLIFLUOR™ probes and dual labeled probe sets.

Please replace the paragraph beginning on page 38, line 2 with the following amended paragraph:

The process for amplification of a desired nucleic acid sequence can be achieve by any means necessary to achieve amplification of the desired amplicon. The amplification can be achieved using any known means in the art, including polymerase chain reaction techniques.

The primers and probes can be purchased or prepared by any means known in the art, including automated processes. In a preferred embodiment, the primers and probes are designed for specificity for the target nucleic acid sequence, as disclosed herein. The enzymes used to promote amplification can be purchased or can be prepared by any means known in the art, including cellular extraction. Substrates to aid in the amplification can also be purchased or can be prepared by any means known in the art, including any synthetic methodology to synthesis natural and unnatural nucleic acids. The enzyme and substrates can be added to the amplification mixture at any time and order that allows for the amplification of the desired amplicon. In a preferred embodiment, the polymerase and substrates follow ~~TaqMan~~ TAQMAN® 7700 chemistry provided by Applied Biosystems in California.

Please replace the paragraph beginning on page 41, line 3 with the following amended paragraph:

Reagent kits that support quantitative amplification and detection in multiplex are commercially available. The QPCR kits are used with DNA templates, either to detect DNA mutations or to measure gene or viral copy number. The QRT-PCR kits are used with RNA templates, typically for measuring RNA levels. Mutations can also be detected in expressed RNA with these kits. These kits have the capability of high performance with various fluorescent detection systems, including, the ~~AmpliFluor~~ AMPLIFLUOR™ system, molecular beacons, ~~TaqMan®~~ TAQMAN® probes, dual fluorophore approach, single-dye primers and DNA binding dyes.

Please replace the title and paragraph beginning on page 41, line 10 with the following amended title and paragraph:

i) ~~Amplifluor~~ AMPLIFLUOR™ Universal Amplification and Detection System, Intergen Co., Purchase, N.Y.

In this system, PCR amplification and detection steps take place in the same reaction vessel. Resultant PCR products fluoresce and can be monitored with real-time or endpoint fluorescence detection instruments. The ~~Amplifluor~~AMPLIFLUOR™ system is based on an innovative adaptation of the molecular beacon technology. Molecular beacons are hairpin-

shaped oligonucleotides that contain fluorophore and quencher moieties. Molecular beacons act like switches that are normally closed to bring the fluorophore/quencher pair together to turn fluorescence "off." When prompted to undergo conformational changes that open the hairpin structure, the fluorophore and quencher are separated, and fluorescence is turned "on." Similarly, the ~~Amplifluor~~AMPLIFLUOR™ system uses a primer that contains a hairpin-shaped end in which fluorescein is paired up with the quencher 4-(dimethylamine)azo benzene sulfonic acid (DABSYL). However, Intergen points out that there is an important difference between the ~~Amplifluor~~AMPLIFLUOR™ system and other currently available energy transfer-based PCR methods (e.g., molecular beacons or Perkin-Elmer's ~~Taqman~~™ TAQMAN®). In ~~Amplifluor~~AMPLIFLUOR™, the fluorescent oligonucleotides are actually incorporated into the reaction products. This enables the *direct* detection of PCR products, reducing the number of false positive reactions, which can be caused by even the most minimal carry-over contamination. Three primers are used to amplify products with Intergen's ~~Amplifluor~~AMPLIFLUOR™ system. Forward and reverse primers specific for the gene of interest are generated by the user. Additionally, reactions contain the ~~UniPrimer~~™ UNIPRIMER™ Energy-Transfer-labeled Primer—the key component of the ~~Amplifluor~~AMPLIFLUOR™ system. The 5' end of ~~UniPrimer~~ UNIPRIMER™ consists of a hairpin structure labeled with fluorescein and DABSYL. A tail sequence (Z) is at the primer's 3' end. The Z sequence acts as a universal PCR primer; it is specifically designed to reduce PCR background due to heterodimer formation. Any PCR reaction can be adapted to the ~~Amplifluor~~AMPLIFLUOR™ system by synthesizing a modified version of one of the target-specific primers (the Z sequence is simply added to the 5' end of the modified primer). Conventional post-PCR detection methods such as gel electrophoresis or dot blot techniques are not required.

Please replace the title and paragraph beginning on page 43, line 4 with the following amended title and paragraph:

(iii) ~~TaqMan~~ TAQMAN® probe.

A cousin of the molecular beacon is the ~~TaqMan~~ TAQMAN® probe from Applied Biosystems of Foster City, Calif. This system exploits the 5' exonuclease activity of Taq DNA

polymerase. During the PCR extension an annealed oligonucleotide that has a reporter fluorophore at the 5' exonuclease and a quencher at the 3' exonuclease is chewed up by a polymerase 5'-3' exonuclease activity, releasing the fluorophore from its quencher (the presence of the ~~TaqMan~~ TAQMAN® probe doesn't significantly inhibit PCR product synthesis). The resulting fluorescence is proportional to the amount of PCR product.

Please replace the title and paragraph beginning on page 44, please replace the title and paragraph beginning on line 1 with the following amended title and paragraph:

(vi) ~~SYBR~~ SYBER® Green I Dye

The fluorescent dye ~~SYBR~~ SYBER® Green I binds to the minor groove of the DNA double helix. In solution, the unbound dye exhibits very little fluorescence, however, fluorescence is greatly enhanced upon DNA-binding. ~~SYBR~~ SYBER® Green I dye is very stable (only 6% of the activity is lost during 30 amplification cycles).

Please replace the paragraph beginning on page 44, line 9 with the following amended paragraph:

After annealing of the primers, a few dye molecules can bind to the double strand. DNA binding results in a dramatic increase of the ~~SYBR~~ SYBER® Green I molecules to emit light upon excitation.

Please replace the paragraph beginning on page 46, beginning on line 6 with the following amended paragraph:

VI. Quantitative Real-Time Polymerase Chain Reaction Using ~~TaqMan~~TAQMAN®

Quantitative real-time polymerase chain reaction using ~~TaqMan~~ TAQMAN®and the Perkin-Elmer/Applied Biosystems division 7700 sequence detector (PE/ABD 7700) provides an accurate method for determination of levels of specific DNA and RNA sequences in samples. It is based on detection of a fluorescent signal produced proportionally during amplification of a PCR product.

Please replace the paragraph beginning on page 48, line 25 with the following amended paragraph:

Several types of reaction mixes are available. The ~~TaqMan~~ TAQMAN® Universal PCR Master Mix, contains the core reagents in an easy to use 2X solution. The ~~TaqMan~~ TAQMAN® Gold RT-PCR kit allows one-step or two-step RT-PCR. The one-step option allows an investigator to set up the RT and PCR steps without opening the tube, whereas the two-step option separates the RT step from the PCR. Master mixes can also be assembled by purchasing the various components, such as NTPs, buffer, Mg^{2+}, and Taq polymerase, from many other companies offering molecular biology reagents.

Please replace the paragraph beginning on page 49, line 3 with the following amended paragraph:

Primers and probes must be carefully designed. The ~~Primer Express~~ PRIMER EXPRESS® software, which is specifically designed to select the primers and probes takes into account the required parameters for well-designed primers and probe. These parameters include a Tm for the probe that is 10°C higher than the primers, primer Tms between 58°C and 60°C, amplicon size between 50 and 150 bases, absence of 5' Gs, and primer length.

Please replace the paragraph beginning on page 51, line 22 with the following amended paragraph:

The ~~TaqMan~~ TAQMAN® probe and primers were designed by using the ~~Primer Express~~ PRIMER EXPRESS® software (Applied Biosystems, CA) and are covering highly conserved sequences complementary to the DNA sequences present in HIV-1 RNA. By scanning the different genotypes of group M for regions containing only minor variability, the conserved domain was discovered. As a result, the region in the HIV-1 RT domain between codon 200 and 280 fulfilled the required criteria; thus this region was used to design an appropriate set of primers and probe that could work in real time PCR ("RT-PCR"). Primer sequences are as follows: sense 5'-TGGGTTATGAACTCCATCCTGAT-3' (Sequence ID No.) and 5'-TGTCATTGACAGTCCAGCTGTCT-3' (Sequence ID No.); the probe sequence is 5'-fluoresent dye-TTTCTGGCAGCACTATAGGCTGTACTGTCCATT-quenching dye-3' (Sequence ID No.). In this particular case, the probe was labeled with FAM at the 5' end, and

the quencher molecule is TAMARA, provided at the 3' end. Any other combination of reporter and quencher dyes can be used as well.

Please replace the paragraph beginning on page 52, line 12 with the following amended paragraph:

The primer and probe set gave a linear range over 6 logs when tested on serial 1-log dilutions of cultured virus. In order to evaluate this primer/probe set with an FDA approved methodology for viral load measurement, a 1-log dilution series of a clinical HIV-1 genotype B isolate (attenuated in vitro to obtain a high viral load) was tested by real time RT-PCR and by Roche ~~Amplicor HIV-1 Monitor~~ AMPLICOR HIV-1 MONITOR™ (**Figure 1**). In this experiment, the 10^{-6} diluted sample became positive at threshold cycle (Ct = 35.52), which corresponded with a 1410 copies/mL in the Roche monitor HIV-1 version II assay. When validated over a dynamic range of 3 logs of virus, there was perfect correlation between the two methodologies (**Figure 1**) with a lower limit of detection for the real-time RT-PCR assay of 141 copies/mL (Ct = 38.85).

Please replace the paragraph beginning on page 52, line 23 with the following amended paragraph:

The real-time RT-PCR technology was evaluated against the NASBA HIV-1 viral load assay. HIV-1 nucleic acid sequences was amplified using the designed probes and primers as described above. Viral RNA present in the culture supernatant was prepared using commercially available columns (~~QIAamp~~ QIAAMP® Viral RNA mini Kit, Qiagen, CA). The amplification reaction mixture was incubated for two minutes at 50°C, then ten minutes at 95°C. Then, the mixture was amplified using forty cycles of a two-step amplification reaction at 95°C for fifteen seconds then sixty seconds at 60°C. Real-time RT-PCR-amplified RNA was detected in real-time by monitoring increases in fluorescence signal that resulted from degradation of a quenched fluorescent probe molecule following to the hybridization of the probe to the amplified viral DNA (~~TaqMan~~ TAQMAN® 7700 chemistry, Applied Biosystems, CA).

Please replace the paragraph beginning on page 54, line 26 with the following amended paragraph:

Several types of reaction mixes are available. The ~~TaqMan~~ TAQMAN® Universal PCR Master Mix, contains the core reagents in an easy to use 2X solution. The ~~TaqMan~~ TAQMAN® Gold RT-PCR kit allows one-step or two-step RT-PCR. The one-step option allows an investigator to set up the RT and PCR steps without opening the tube, whereas the two-step option separates the RT step from the PCR. Master mixes can also be assembled by purchasing the various components, such as NTPs, buffer, Mg2+, and Taq polymerase, from many other companies offering molecular biology reagents.

Please replace the paragraph beginning on page 55, line 5 with the following amended paragraph:

Primers and probes must be carefully designed. The ~~Primer Express~~ PRIMER EXPRESS® software, which is specifically designed to select the primers and probes takes into account the required parameters for well-designed primers and probe. These parameters include a Tm for the probe that is 10°C higher than the primers, primer Tms between 58°C and 60°C, amplicon size between 50 and 150 bases, absence of 5' Gs, and primer length.

Please replace the paragraph beginning on page 59, line 14 with the following amended paragraph:

HIV-1 particles were brought into culture using human PBM cells. Viral RNA present in the culture supernatant was prepared using commercially available columns (~~QIAamp~~ QIAAMP® Viral RNA mini Kit, Qiagen, CA). RT-PCR–amplified RNA was detected in real-time by monitoring increases in fluorescence signal. A total of 5 μL RNA was RT-amplified using reagents and conditions as described by the manufacturer (Applied Biosystems, CA). The standard curve ranged from 1.41×10^2 copies/mL to over 1.41×10^8 copies/mL. Copy numbers were calibrated using the Roche ~~Amplicor HIV-1 Monitor test™~~ AMPLICOR HIV-1 MONITOR™ (Roche Diagnostics, Branchburg, NJ), or the NASBA HIV-1 viral load assay (Organon Technika). Correlation coefficient is in all experiments greater than 0.99. **(Figure 1).**

Please replace the paragraph beginning on page 60, line 3 with the following amended paragraph:

As of today, the only reliable and available system for HCV RNA replication is the replicon system in Huh7 cells. The cells were brought into culture for several days and total RNA present in the culture was prepared using commercially available columns (~~QIAamp~~ QIAAMP® Viral RNA mini Kit, Qiagen, CA). RT-PCR–amplified RNA was detected in real-time by monitoring increases in fluorescence signal. A total of 5 μL RNA was RT-amplified using reagents and conditions as described by the manufacturer (Applied Biosystems, CA). The standard curve ranged from 45 IU/mL to over 4.7 x 10^7 IU/mL. Copy numbers were calibrated using the Roche ~~Amplicor HIV-1 Monitor test™~~ AMPLICOR HIV-1 MONITOR™ (Roche Diagnostics, Branchburg, NJ). Correlation coefficient is in all experiments greater than 0.99 **(Figure 2).**

Please replace the paragraph beginning on page 60, line 14 with the following amended paragraph:

HBV viral particles are released from at leasts three different cell lines: HepG2.2.1.5, HEPAD38 and HepAD79 cell lines. The cells were brought into culture for several days and total nucleic acids present in the culture supernatant, or in the cells, was prepared using commercially available columns (~~QIAamp~~ QIAAMP® Viral RNA mini Kit, Qiagen, CA). PCR–amplified DNA was detected in real-time by monitoring increases in fluorescence signal. A total of 5 μL DNA was RT-amplified using reagents and conditions as described by the manufacturer (Applied Biosystems, CA). The standard curve ranged from 2 copies to over 2 x 10^7 copies per reaction mix. Copy numbers were calculated ~~form~~ from OD260 values obtained from an HBV standard. Correlation coefficient is in all experiments greater than 0.99.

Please replace the paragraph beginning on page 61, line 3 with the following amended paragraph:

BVDV viral particles are released from infection experiments using the strain NADL on MDBK cells (both available ~~form~~ from ATTC). After infection, the cell were brought into culture for several days and total nucleic acids present in the culture supernatant, or in the cells, was prepared using commercially available columns (~~QIAamp~~ QIAAMP® Viral RNA mini Kit, Qiagen, CA). RT-PCR–amplified RNA was detected in real-time by monitoring increases in fluorescence signal. A total of 5 μL DNA was RT-amplified using reagents and conditions as

described by the manufacturer (Applied Biosystems, CA). The standard curve ranged from 0.6 plaque forming units to over 6 x 10^3 plaque forming units per reaction mix. Plaque forming units were calculated form traditional plaque assays. Correlation coefficient is in all experiments greater than 0.99.

Please replace the paragraph beginning on page 61, line 16 with the following amended paragraph:

RSV viral particles are released from infection experiments using the available virus strain derived from a clinical sample on A549 or Hep2 cells. After infection, the cell were brought into culture for several days and total nucleic acids present in the culture supernatant, or in the cells, was prepared using commercially available columns (~~QIAamp~~ QIAAMP® Viral RNA mini Kit, Qiagen, CA). RT-PCR–amplified RNA was detected in real-time by monitoring increases in fluorescence signal. A total of 5 µL DNA was RT-amplified using reagents and conditions as described by the manufacturer (Applied Biosystems, CA). The standard curve ranged from 70 plaque forming units to over 7 x 10^3 plaque forming units/mL. Plaque forming units were calculated form traditional plaque assays. Correlation coefficient is in all experiments greater than 0.99. Hep2 cells gave the highest virus titer after 72 hours of incubation, the amount of cells used varied between 10,000 and 50,000 cells per well, but there were no differences observed in total amount of virus production at 72 hours.

Please replace the paragraph beginning on page 62, line 7 with the following amended paragraph:

As one illustration of this method, in a first step, HepG2 cells are kept in culture in presence of 10 microMolar of a set of candidate antiviral agents. Subsequently, total DNA is isolated from cultured HepG2 cells by means of a commercially available columns (~~QIAamp~~ QIAAMP® DNA Blood Mini Kit, Qiagen, CA). Total DNA was eluted from columns in 200 µL water. The mitochondrial gene and nuclear gene are then amplified with a quantitative real-time PCR protocol using the suitable primers and probes. Reagents and conditions used in quantitative PCR were purchased from PE-Applied Biosystems.

Please replace the paragraph beginning on page 63, line 26 with the following amended paragraph:

Total DNA is isolated from cultured HepG2 cells by commercially available columns (~~QIAamp~~ QIAAMP® DNA Blood Mini Kit, Qiagen, CA). Total DNA was eluted from columns in 200 µL of water. The mitochondrial gene and nuclear gene are then amplified with a quantitative real-time PCR protocol using suitable primers and probes. A set of primers and fluorescent probes for both nuclear and mitochondrial DNA or RNA was designed; the endogenous control DNA primer set is given by 5'-GCG CGG CTA CAG CTT CA-3' (Sequence ID No.) and 5'-TCT CCT TAA TGT CAC GCA CGA T-3' (Sequence ID No.); the mitochondrial DNA primer set is given by 5'-TGC CCG CCA TCA TCC TA-3' (Sequence ID No.) and 5'-TCG TCT GTT ATG TAA AGG ATG CGT-3' (Sequence ID No.). The probe for nuclear gene is given by 5'-fluorescent Dye-CAC CAC GGC CGA GCG GGA-fluorescent quencher-3' (Sequence ID No.); fluorescent labeled probes for mitochondrial genome is given by 5'-fluorescent Dye-TCC TCA TCG CCC TCC CAT CCC-fluorescent quencher-3' (Sequence ID No.). Reagents and conditions used in quantitative PCR were purchased from PE-Applied Biosystems.

Please replace the paragraph beginning on page 72-73, bridging paragraph, with the following amended paragraph:

As one illustration of this method, in the case of BVDV in MDBK cells, in a first step, viral RNA is isolated from 140 µL of the cell culture supernatant by means of a commercially available column (Viral RNA extraction kit, QiaGen, CA). The viral RNA is then eluted from the column to yield a total volume of 60 µL, and subsequently amplified with a quantitative RT-PCR protocol using a suitable primer for the BVDV NADL strain. A quenched fluorescent probe molecule is hybridized to the BVDV DNA, which then undergoes exonucleolytic degradation resulting in a detectable fluorescent signal. Therefore, the RT-PCR amplified DNA was detected in real time by monitoring the presence of fluorescence signals. The ~~TaqMan~~ TAQMAN® probe molecule (5'-6-FAM-AAATCCTCCTAACAAGCGGGTTCCAGG-TAMRA 3' [Sequence ID No] and primers (sense: 5'-AGCCTTCAGTTTCTTGCTGATGT-3' [Sequence ID No]; and antisense: 5'–TGTTGCGAAAGCACCAACAG-3' [Sequence ID No]) were designed with the aid of the ~~Primer Express~~ PRIMER EXPRESS® software (PE-Applied

Biosystems) to be complementary to the BVDV NADL NS5B region. A total of 10 μL of RNA was analyzed in a 50 μL RT-PCR mixture. Reagents and conditions used in quantitative PCR were purchased from PE-Applied Biosystems. The standard curve that was created using the undiluted inoculum virus ranged from 6000 plaque forming units (PFU) to 0.6 PFU per RT-PCR mixture. A linear range of over 4-logs was routinely obtained.